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खाद्य उत्पादों के लिये अन्नाटो रंग — विशिष्टि

(पहला पुनरीक्षण)

Indian Standard

ANNATTO COLOUR FOR FOOD PRODUCTS — SPECIFICATION

(First Revision)

Second Reprint SEPTEMBER 2001

UDC 633·863·92 : 637·2/·3

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

October 1994

Price Group 2

AMENDMENT NO. 1 FEBRUARY 2006
TO
IS 2557 : 1994 ANNATTO COLOUR FOR FOOD PRODUCTS — SPECIFICATION

(First Revision)

(*Page* 1, *clause* 4.2.1) — Substitute the following for the existing clause:

'Annatto extract in oil, as solution or suspension, is prepared by extraction of the outer coating of seeds with vegetable oils. In the preparation of the solution of annatto colour in oil, only the vegetable oils included under the *Prevention of Food Adulteration Rules*, 1955 shall be used, either singly or in a mixture and shall conform to relevant Indian Standards where it exists.'

(FAD 8)

Reprography Unit, BIS, New Delhi, India

AMENDMENT NO. 2 APRIL 2011
TO
IS 2557 : 1994 ANNATTO COLOUR FOR FOOD PRODUCTS — SPECIFICATION

(First Revision)

[*Page* 3, *clause* **6.2.1**(c))] — Substitute 'net fluid quantity' *for* 'net fluid contents'.

(*Page* 4, *clause* **B-1.1**) — Substitute the following for the existing formula:

$$\text{Percent total carotenoid (expressed as bixin)} = \frac{A}{2.826} \times \frac{1\ 000\ 000}{\text{Sample mass (mg)} \times 100}$$

(*Page* 4, *clause* **B-2.1**) — Substitute the following for the existing formula:

$$\text{Percent total carotenoid (expressed as norbixin)} = \frac{A}{3.473} \times \frac{1\ 000\ 000}{\text{Sample mass (mg)} \times 100}$$

(FAD 8)

Reprography Unit, BIS, New Delhi, India

Food Additives Sectional Committee, FAD 8

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council.

Annatto colour is obtained from the aril (coating of the seed) commonly known in commerce as the seed of the plant *Bixa orellana* L. Its solution in oil is used for colouring butter and other food products while its solution in water with a little alkali is used for colouring cheese and other similar products.

This standard is one of a series of Indian Standards on natural food colours permitted under the *Prevention of Food Adulteration Rules*, 1955 with the latest amendments, issued by the Ministry of Health, Government of India. In the preparation of this standard due consideration has been given to these Rules. Due consideration has also been given to the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977. However, this standard is subject to the restriction imposed under these Rules wherever applicable.

This standard was first published in 1963. It has been revised taking into account the latest technological developments in the country and also to bring it in line with the International specifications. In this revision minimum limits for carotenoids and maximum limits for heavy metals have been included as well as identification tests for annatto.

In the preparation of this standard, assistance has been derived from the following publications.

> Specification for Identity and Purity of Food Additives, Food and Nutrition Paper 19, published by Food and Agriculture Organization of the United Nations and the World Health Organization.
>
> Food Chemical Codex published by the National Academy of Sciences and National Research Council, Washington DC, USA. Second supplement of 1981 Edition.

Description

Class — Carotenoids

Code Number — CI (1975) No. 75120
CI (1975) Natural Orange 4
EEC No. E-160 b

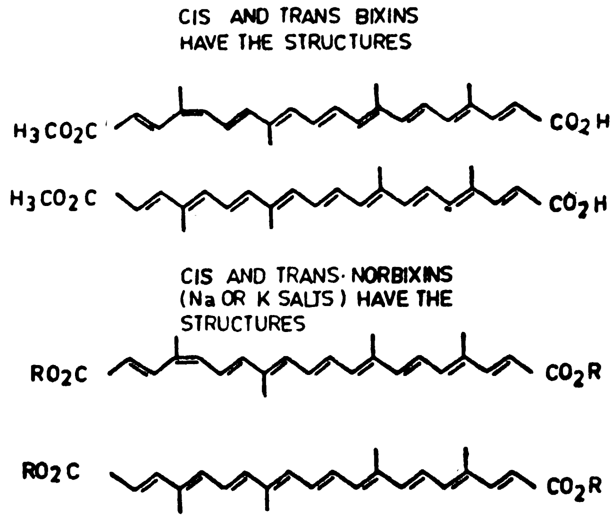
Chemical Name

Annatto extract in oil contains several coloured components, the major single one being bixin, which may be present in both *Cis* and *trans* forms. Thermal degradation products of bixin may also be present.

Water soluble annatto contains norbixin, the hydrolysis product of bixin, in the form of sodium or potassium salt, as the major colouring principle. Both *Cis* and *trans* forms may be present.

Chemical Formula — Bixin $C_{25}H_{30}O_4$
Norbixin $C_{24}H_{28}O_4$

Structural Formula



where R = Na or K

(*Continued on third cover*)

(*Continued from second cover*)

Molecular Weight — Bixin 394·50
Norbixin 380·48

For the purpose of deciding whether a particular requirement of this standard is complied with, final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number significant places retained in the rounded off value should be the same as that of the specified value this standard.

Indian Standard
ANNATTO COLOUR FOR FOOD PRODUCTS — SPECIFICATION
(First Revision)

1 SCOPE

1.1 This standard prescribes the requirements and the methods of test for annatto colour derived from the plant *Bixa orellana* L. and supplied in the form of (a) solution in oil, and (b) solution in water, for use in butter, cheese and other food products.

2 REFERENCES

The Indian Standards listed at Annex A are necessary adjuncts to this standard.

3 TYPES

3.1 The material shall be of the following two types:

a) Solution in oil for use in butter and other food products, and
b) Solution in water for use in cheese and other food products.

4 REQUIREMENTS

4.1 General

The material shall be derived only from the plant *Bixa orellana* L. and shall not contain any extraneous colouring matter. It shall be processed, packed, stored and distributed under hygienic conditions in licenced premises (*see* IS 2491 : 1972).

4.2 Solution of Annatto Colour in Oil for Use in Butter and Other Food Products

4.2.1 Annatto extract in oil, as solution or suspension, is prepared by extraction of the outer coating of seeds with vegetable oils. In the preparation of the solution of annatto colour in oil, only the vegetable oils included under the *Prevention of Food Adulteration Rules, 1955* shall be used, either singly or in a mixture and shall conform to the following specifications:

a) Coconut oil – conforming to IS 542 : 1968
b) Groundnut oil – conforming to IS 544 : 1968
c) Mustard oil – conforming to IS 546 : 1975
d) Sesame oil – conforming to IS 547 : 1968
e) Linseed oil – conforming to IS 75 : 1973
f) Nigerseed oil – conforming to IS 3490 : 1965
g) Safflower seed oil – conforming to IS 3491 : 1965
h) Olive oil – conforming to specifications given under Indian Pharmacopoeia
j) Poppy seed oil – conforming to specifications given under *Prevention of Food Adulteration Rules, 1955.*

4.2.2 The solution of annatto colour in oils shall be clear and shall remain so on storage in suitable containers at 15°C except for a slight deposit of stearine or shall be in the form of a suspension. The suspension on dilution with hot oil to bring the bixin content to 0.24 percent shall be a clear solution.

4.2.3 *Colour*

The colour of solution in amyl acetate (*see* IS 231 : 1957) at a dilution of 1 : 1 000 (*w/v*), when measured in a Lovibond Tintometer with a 1-cm cell, shall be not less than the following:

Yellow units	5.0
Red units	0.4

or be not less than the colour of the following inorganic solution at a liquid depth of one centimetre which may be employed for matching the stated dilution in a plunger type colorimeter using incident light closely approximating the north day light:

Potassium bichromate	0.320 g
Cobalt ammonium sulphate $[CoSO_4, (NH_4)_2 SO_4, 6H_2O]$	2.02 g
Sulphuric acid sp-gr 1.84	2 ml
Distilled water	To make solution to one litre

These reagents shall be of the analytical reagent grade (*see* IS 250 : 1964 and IS 266 : 1993). Although the solution retains its tinctorial value for a considerable time, after prolonged storage, its optical clarity shall be examined before use, to ensure that no alteration has taken place.

NOTES

1 Diluted solution of annatto colour in amyl acetate is not stable in colour quality particularly if exposed to light, and measurement should be carried out on the diluted solution without undue delay.

2 In using the Tintometer, the maker's instructions shall be rigidly followed.

4.3 Solution of Annatto Colour in Water for Use in Cheese and Other Food Products

4.3.1 Water soluble annatto is prepared by extraction of the outer coating of the seeds with aqueous alkali (sodium or potassium hydroxide). In the preparation of the solution, potable water shall be used. A little quantity (0.5 to 3 percent) of alkali may be added (*see* IS 376 : 1986 and IS 296 : 1986).

4.3.2 The solution shall be clear and shall remain so on storage in suitable containers at a temperature of 15°C.

4.3.3 *Colour*

The colour of the solution in 0.1 N sodium hydroxide or potassium hydroxide at a dilution 1 : 1 000 *(m/v)* measured in a 1-cm cell shall be the same as that specified in 4.2.3.

4.4 Identification

4.4.1 *Spectrophotometry*

Annatto extracts in oil, diluted with chloroform exibits absorbance maxima at 439, 470 and 501 nm. Water soluble annatto extracts, diluted with water exibit absorbance maxima at 453 and 482 nm.

4.4.2 *Column Chromatography and Carr-Price Reaction*

4.4.2.1 *Preparation of alumina column*

Prepare a small chromatography column by filling a glass tube (7 x 200 mm internal diameter), stoppered with glass wool, with alumina (800 - 200 mesh) slurried in benzene so that the settled alumina fills about two-thirds of the tube. Fill the column with benzene and adjust the flow rate to about 30 drops/min using a rubber outlet tube and clamp until the benzene layer is 2-3 mm above the alumina surface.

NOTE - It is advisable to place a glass-wool plug at the top of the alumina to prevent churning when solvent is added, and to apply a reservoir of solvent at the top of the column, which will exert a mild pressure on the chromatographic column. The suction must be carefully controlled to prevent channelling in the alumina. Avoid eluting the benzene or other solvent completely. It is neccessary that the surface of the alumina is kept covered with liquid layer.

4.4.2.2 *Annatto extract in oil*

Add to the top of the alumina column 3 ml of a solution containing sufficient sample, in benzene, to impart a colour equivalent to a 0.1% potassium dichromate solution; elute with benzene until a pale yellow fraction is washed from the column. Wash the column with three 10-ml volumes of dry chloroform; then add 5 ml of Carr-Price reagent and allow it to run onto the top of the column. The orange-red zone (bixin) at the top of the column immediately becomes blue-green.

4.4.2.3 *Water soluble annatto*

Transfer 2 ml or 2 g to a 50-ml separatory funnel and add sufficient 2 N sulphuric acid to make the solution acidic to *p*H test paper (*p*H 1-2). Dissolve the red precipitate of norbixin by mixing the solution with 50 ml of benzene. Discard the water layer and wash the benzene phase with water until it no longer gives an acid reaction. Remove any undissolved norbixin by centrifugation or filtration and dry the solution over anhydrous sodium sulphate. Transfer 3-5 ml of the dry solution to the top of an alumina column prepare as described above. Elute the column with benzene, three 10-ml volumes of dry chloroform, followed by 5 ml of Carr-Price reagent added to the top of the column. The orange-red band of norbixin immediately becomes blue-green.

4.4.3 *Thin Layer Chromatography*

4.4.3.1 *Annatto extract in oil*

Use silica gel with 12 percent calcium sulphate as binding agent, and a mixture of acetic acid, chloroform and acetone (1 : 50 : 50, by volume) as developing solvent. The sample in oil produces at least 3 or 4 red or yellow spots. Two red spots are clearly more intense than the other spots. Dissolve the two spots in benzene and identify the spot that is bixin by the method given in 4.4.2.2.

4.4.3.2 *Water soluble annatto*

Prepare a chromatogram as indicated under 4.4.3.1. Three or four spots are obtained, of which 2 spots have an orange colour and the others an orange-yellow colour. Dissolve the orange spots in benzene and identify one of the spots that is norbixin by the method given in 4.4.2.3.

5 PURITY TESTS

5.1 The material shall conform to the requirements given in Table 1.

Table 1 Requirements for Annatto

(*Clause* 5.1)

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Annex of This Standard	Ref to Clause of IS 1699 : 1994
(1)	(2)	(3)	(4)	(5)
i)	Carotenoids			
	a) Annatto extract in oil, expressed as bixin, percent by mass, *Min*	0.24	B	-
	b) Water-soluble annatto, expressed as norbixin, percent by mass, *Min*	0.24	B	-
ii)	Arsenic, mg/kg, *Max*	3	-	15
iii)	Lead, mg/kg, *Max*	10	-	15
iv)	Copper mg/kg, *Max*	30	-	15
v)	Heavy metals, mg/kg, *Max*	40	-	16

6 PACKING, MARKING AND STORAGE

6.1 Packing

The material shall be filled in suitable containers with as little air space as possible so as to preclude contamination of the contents with metals or other impurities, and should preferably exclude light.

6.2 Marking

6.2.1 Each container shall be marked legibly and indelibly to give the following information:

a) Name and type of the material;
b) Source of manufacture;
c) Minimum net fluid contents;
d) Batch or code number;
e) Date of manufacture;
f) Declaration of bixin and norbixin content;
g) A statement to the effect that the material should be kept in a cool place, and in the case of glass containers, away from light; and
h) Any other details as specified under the *Standards of Weights and Measures (Packaged Commodities) Rules, 1977/Prevention of Food Adulteration Rules, 1955.*

6.2.2 *BIS Certification Marking*

The product may also be marked with the Standard Mark.

6.2.2.1 The use of the Standard Mark is governed by the provisions of the Bureau of Indian Standards Act, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6.3 Storage

The material shall be stored in a cool place.

7 SAMPLING

7.1 Representative samples of the material for the tests shall be drawn according to the method prescribed in 4 of IS 1699 : 1994.

8 TESTS

8.1 Tests shall be carried out as prescribed in col 4 and 5 of Table 1.

8.2 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070 : 1992) shall be employed in tests.

NOTE – 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

ANNEX A

(*Clause* 2)

LIST OF REFERRED INDIAN STANDARDS

IS No.	*Title*
75 : 1973	Linseed oil, raw and refined (*second revision*)
231 : 1957	Amyl acetate
250 : 1964	Potassium bichromate, technical and analytical reagent (*revised*)
266 : 1993	Sulphuric acid (*third revision*)
296 : 1986	Sodium carbonate. anhydrous (*third revision*)
376 : 1986	Sodium hydroxide, analytical reagent (*third revision*)
542 : 1968	Coconut oil (*second revision*)
544 : 1968	Groundnut oil (*second revision*)
546 : 1975	Mustard oil (*second revision*)
547 : 1968	Seasame oil (*second revision*)
1070 : 1992	Reagent grade water (*third revision*)
1699 : 1994	Methods of sampling and test for food colours (*second revision*)
2491 : 1972	Code for hygienic conditions for food processing units (*first revision*)
3490 : 1965	Nigerseed oil
3491 : 1965	Safflower oil

ANNEX B

[*Table 1, Item* (i)]

DETERMINATION OF CAROTENOIDS

B-1 ANNATTO EXTRACT IN OIL

B-1.1 Transfer 0.1 to 1 g of the sample, accurately weighed, into a 100-ml volumetric flask. Dissolve in chloroform, dilute to volume with chloroform, and mix. Transfer a 1-ml portion of the solution into another 100-ml volumetric flask, and dilute to 100 ml.

Measure the absorbance A of this solution at the wavelength of 470 nm.

$$\text{Percent total carotenoid (expressed as bixin)} = \frac{A}{2.826} \times \frac{100\,000}{\text{Sample mass (mg)} \times 100}$$

B-2 WATER-SOLUBLE ANNATTO

B-2.1 Weigh accurately 0.1 to 1 g of the sample, add 0.01 N sodium hydroxide to 100 ml, and shake thoroughly. Transfer 1 ml portion of the solution into a separating funnel, add 10 ml of sodium chloride solution (1 in 10) and water to 50 ml, and add 2 ml of diluted sulphuric acid. Continue to extract the solution with each 10 ml of benzene until the benzene extract is not coloured. Combine benzene extract, wash three times with each 5 ml of water, and allow to stand to separate the water layer. Transfer the benzene extract into another separating funnel containing the water layer three times with 2 ml of benzene, and combine the washings to the benzene extract. To the benzene extract, add an equal volume of petroleum benzene mix, and continue to extract with each 5 ml of 0.01 N sodium hydroxide until sodium hydroxide solution is not coloured. Combine extracts, and add 0.01 sodium hydroxide to 100 ml. Measure the absorbace A at the wavelength of 453 nm.

$$\text{Percent total carotenoid (expressed as norbixin)} = \frac{A}{3.473} \times \frac{100\,000}{\text{Sample mass (mg)} \times 100}$$

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This Indian Standard has been developed from Doc : No. FAD 8 (256).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

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Printed at New India Printing Press, Khurja, India